Extracellular Matrix Surrogates for Tumoroid Generation

Viren Sardana[#], Neha Arya[#], D. S. Katti[*]
Department Biological Sciences and Bioengineering, IIT Kanpur, India-208016

Statement of Purpose: *In vitro* study of tumor biology has been majorly restricted to conventional two-dimensional petridish culture and so is the case for cancer related drug screening and toxicology studies. However, the conventional cell culture techniques (*in-vitro* systems including matrigel^{TM}) provide only suboptimal mimicking of the *in vivo* environment primarily because they lack the right combination of cues essential for the recreation of an *in-vivo* tumor environment [1, 2]. Further, no single animal model for cancer is the exact surrogate for human disease and its multiple patterns. Therefore, it will be useful to generate *in vitro* tumor models that can represent the complexities of real tumor environment. In this study, we attempt to generate physical surrogates of extracellular matrix of solid tumors, which can provide a microenvironment to cancer cells that may enable formation of tumoroids. In this regard, we have generated polymeric scaffolds of extracellular matrix mimics – gelatin (collagen mimic) and chitosan (glycosaminoglycan mimic), in different ratios with genipin as the cross-linker. The scaffolds were characterized for their physical properties and their ability to support tumoroid generation.

Methods: Chitosan (viscosity <200 mPa.s) and gelatin (Type A 300 Bloom) were used in ratios of 1:2, 1:1 and 2:1 (chitosan:gelatin :: C:G) w/w and cross-linked with genipin (0.75% w/w of polymer) to form 3-D scaffolds by the freeze drying technique. The scaffolds were prepared at varying freezing temperatures of -20°C and -80°C and characterized for their morphology by scanning electron microscopy (SEM), and for percentage total pore volume, degree of cross-linking, swelling kinetics, water absorption capacity and functional group analysis by FTIR. NCI-H460 cells (a non-small cell lung cancer line) were seeded onto the scaffolds and incubated under static conditions for 9 days. Viability assay (MTT) was performed at the end of 3rd, 6th, and 9th day.

Results: SEM imaging revealed differences in pore size in scaffolds fabricated at -20°C (larger) and -80°C (smaller) (**Figure 1**). Total pore volume percentage determined by cyclohexane for scaffolds generated at -80°C was 95.53% and for -20°C was 94.37%. Scaffolds produced at both freezing temperatures showed no significant difference in water absorption capacity (swelling) at the end of 10 minutes. FTIR revealed increase in the relative ratio of intensities of the absorption bands at 1640–1660 cm^{-1} (amide C=O stretching vibrations) and at 1550–1560 cm^{-1} (amine and amide N–H bending vibrations) in cross-linked samples as compared to that of un-cross-linked samples indicating formation of new amide bonds due to cross-linking. Ninhydrin assay was performed to assess difference in cross-linking amongst scaffolds and there was no significant difference in absorbance at 570 nm indicating that the degree of cross-linking was approximately the same. *In vitro* cell culture studies were performed to understand the ability of scaffolds to support the formation of tumoroids.

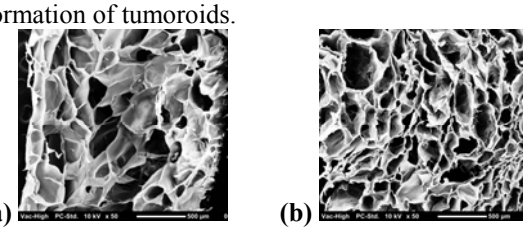

Figure 1. Transverse section of chitosan-gelatin scaffolds generated by freeze drying at (a) -20 °C and (b) -80°C

A significant increase in cell proliferation and viability was observed (**Figure 2**) in majority of scaffolds (disc shaped; 1 mm thick) from day 3 to day 9 (P<0.01). Cell viability (done for scaffolds with C:G :: 1:2 at -80°C) decreased with the increase in scaffold thickness from 1 mm to 3 mm which may be attributed to limited cellular infiltration in the thicker scaffolds (P<0.01) (**Figure 3**). NCI-H460 cells were seeded onto the scaffolds and tumoroid like structures (**Figure4**) were observed for majority of the scaffolds.

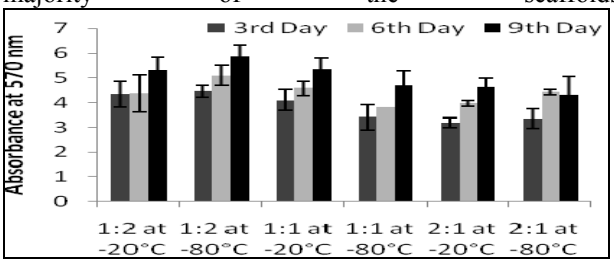

Figure 2. Cell viability on scaffolds with chitosan : gelatin ratio of 1:2, 1:1, 2:1 using freezing temperature of -20°C or -80°C (scaffold thickness 1 mm)

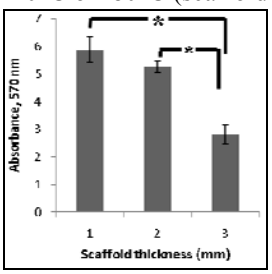

Figure 3. Comparison of cell viability on scaffolds with different thicknesses (*P<0.01)

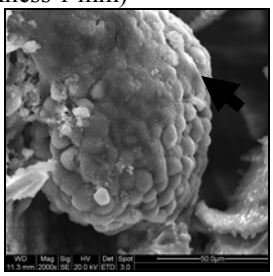

Figure 4. SEM image of NCI-H460 tumoroid like structure after 7 days of in vitro culture.

Conclusions: Chitosan-gelatin scaffolds were successfully synthesized by the freeze drying method and results demonstrate that scaffolds with 1 mm thickness and having higher concentration of gelatin led to higher cell numbers with time and tumoroid like structure formation. Therefore, these scaffolding systems show potential to be developed as *in vitro* tumor models for studying cancer biology / applied chemotherapeutics.

References: (1). Cukierman E. Science. 2001; 294:1708-1712. **(2)** Smalley K.S.M. In Vitro Cell. Dev.Biol. 2006;42. 242-247.

# Contributed equally, * Corresponding author